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Cross and co resistance among Danish porcine *E. coli* isolates

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Abstract

Cross and co-resistance to antimicrobials are presented for 765 Danish *Escherichia coli* isolates of porcine origin from 2009-2013. All isolates and data originate from the DANMAP surveillance but have not previously been used to describe the occurrence of cross and co- resistance. Data presented here clearly indicate the ability of low classified antimicrobials as ampicillin to uphold resistance to critical important antimicrobials for human treatment.

Keywords

Co resistance, cross resistance, multi resistance, *E. coli*, ampicillin

It is generally believed that prevalence of antimicrobial resistance is a result of usage of antimicrobial (Levy 1997). This usage will select for already existing resistance bacteria enhancing their prevalence. General observations of prevalence of resistance have in contradiction to this shown non linear relationship. Major changes of usage can occur while the prevalence of antimicrobial resistance prevails (Aarestrup 1999). Co - and cross resistance mechanisms are most likely responsible for this observation (Pitout and Laupland 2008). In general, surveillance data is not presented in ways that visualize this (Anonymous 2014) and the European Food safety Authority experts have lately suggested that analysis of surveillance data should include clear structured tables that visualize co - and cross resistance (EFSA 2012).

Here we analyze data for 765 porcine *E. coli* isolates obtained from 2009-2013 as part of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) (Table 1) to investigate for presence of cross- and co-resistance. Isolates were collected from fecal samples collected at slaughterhouses in Denmark each year. Single isolates representing farms in Denmark were isolated by first directly plaiting on Drigalski agar, then picking yellow colonies, incubating these in BBL CHROM agar Orientation Medium and isolated as red colonies from this media after o.n. incubation at 37 °C. For more details see Anonymous 2014.

Isolates were tested for antimicrobial resistance to 16 compounds, representing eight distinct antimicrobial structural families. The compounds include tetracyclines, β-lactams, sulphonamides, trimethoprim, aminoglycosides, amphenicols, quinolones and polymyxins (colistin) (Anonymous 2014) representing antimicrobial classes, which WHO has classified according to their importance for treatment of human infections (Collignon et al. 2009). Resistance was defined according to EUCAST epidemiological cut-off values and antimicrobial resistance testing was done as microbroth dilution MIC with Sensititre and inoculation procedures according to CLSI guidelines and European standard (ISO 20776-1:2006)(Anonymous 2014)

Co-resistance was defined as resistance to different antimicrobial compounds belonging to the same chemical structure group (β-lactams, aminoglycosides, amphenicols and quinolones). Co-resistance can be encoded by one resistance determinant. Cross-resistance is defined as resistance to two or more compounds belonging to different distinct antimicrobial resistance compound and will be encoded by series of antimicrobial resistance determinants.

Overall 45% (n=334) of the tested isolates were fully susceptible to all antimicrobials tested and 32% (n=245) were found multi-resistant using the EFSA definition of resistance to more than five antimicrobial families (EFSA 2012),. Resistance profiles were sustained in all tested years. The most dominant multi-resistant profile was resistance to ampicillin (AMP), streptomycin (STR), sulphonamides (SUL), tetracycline (TET) and trimethoprim (TRI); 6% (n=48) of the isolates had this profile. 27% of all isolates (n=205) were resistant to β-lactams. All cephalosporin-resistant (tested with the 3rd generation cephalosporins cefotaxime and ceftiofur) isolates (n=5) had cross-resistance to ampicillin due to genetic background. Two percent of the ampicillin (n=205) resistant isolates were cross-resistance to cephalosporins.

Forty-two percent of all isolates (n=320) where resistance to streptomycin. Among aminoglycoside-resistant isolates, resistance is encoded by numerous resistance genes (Shaw et al. 1993), all giving different patterns for cross-resistance (Hedges and Shannon 1984). 92% of the spectinomycin-resistant isolates (n=159), 85% of the neomycin-resistant isolates (n=34), 46% of the spectinomycin (n=147) and 9% of the neomycin (n=9) resistant isolates showed cross-resistance to streptomycin. All apramycin-resistant isolates (n=3) and gentamicin-resistant isolates (n=5) were cross-resistant to streptomycin due to genetic background for resistance. This has been reported previously (Hedges and Shannon 1984, Sandvang and Aarestrup 2000).

Less than one percent of all tested isolates were resistant to amphenicols but due to common genetic background for resistance all florfenicol-resistant *E. coli* isolates (n=4) had cross-resistance to chloramphenicol. However, only 12 % of the chloramphenicol-resistant *E. coli* isolates (n=34) showed cross-resistance to florfenicol.

Less than one percent of the tested isolates (n=5) were quinolone-resistant but due to a shared genetic background for antimicrobial resistance, cross-resistance was observed between resistance to nalidixic acid and ciprofloxacin (n=5).

The highest levels of co-resistance were found among isolates resistant to sulphonamides (five distinct structural groups). Among those isolates 100 % of the quinolone-resistant isolates (n=5), 91-100% of the amphenicols (chloramphenicol (91%, n=34) and florfenicol (100%, n=4)) resistant isolates, 91-100% of amphenicols (91% chloramphenicol (n=34) and 100% florfenicol (n=4)) resistant isolates, 93% of trimethoprim (n=170) resistant isolates, 80-91% of the aminoglycoside (gentamicin (80%, n=5) and neomycin (91%, n=34)) resistant isolates and 80-85% of the β-lactams (ampicillin (85%, n=205) and 3rd generation cephalosporin (80%, n=5)) resistant isolates were co-resistant to sulphonamides.

High co-resistance was furthermore found among isolates resistant to streptomycin (four families), tetracycline (two families), ampicillin (two families) and trimethoprim (one family).

Surprisingly, results presented here shows that while 93% of the sulphonamides-resistant isolates were co-resistant to trimethoprim only 63 % of trimethoprim resistant isolates were co-resistant to sulphonamides. These two antimicrobials are often used in combination (Anonymous 2014), and therefore, an equal prevalence of co-resistance was expected and even though encoded by difference resistance genes are often found together on plasmids (Freitag et al. 2017). No explanation for this can be given.

Co- and cross-resistance could cause the observed disproportions between the prevalence of resistance and the quantitative usage of some of the antibiotics (Anonymous 2014). Co- and cross-resistance to commonly used antimicrobials, such as tetracycline, ampicillin, sulphonamides and streptomycin, may thus uphold resistance to other chemically distinct antimicrobial families. In accordance to this, usage of antimicrobials not listed as critically important for human treatment can uphold already developed resistance to critically important antimicrobials, as well as selecting multi-resistant profiles otherwise limiting treatment possibilities. Co-resistance can thus be the driver for presence of resistance to the critical important antimicrobials for human treatment in *E.*

coli in Danish pigs several years after the industry has stopped using these antimicrobials, making it extremely difficult to eliminate prevalence of unwanted antimicrobial resistance. Surprisingly, all isolates resistant to 3rd generation cephalosporins, fluoroquinolones and gentamicin were resistant to ampicillin. Cross-resistance between ampicillin and 3rd generation cephalosporins is not surprising, but co-resistance to quinolones and the aminoglycosides apramycin/gentamicin was not expected. This result could indicate that the usage of ampicillin can uphold already acquired resistance to these critical important antimicrobials. The same level of co-and cross resistance between tetracycline and other critical antibiotics was not observed indicating that usage of ampicillin potentially could be more important for upholding critical resistance in pig production in Denmark than tetracycline (Anonymous 2014). Most of the isolates containing resistance to important antimicrobials are multi-resistant leaving few options to eliminate infections. It was also observed that isolates with specific multi-resistant profiles persisted over the study period and made up a fair proportion (32%) of the 765 randomly picked *E.coli* isolates from pigs. Distinct resistance profiles, such as AMP-STR-SUL-TET-TRI (6%, n=9) prevailed during the whole period. A closer review of all resistance profiles reveals a backbone profile (AMP-STR-SUL) found in 20% of the isolates (151 of 765 isolates). This combination is found in almost all multi-resistant isolates showing the addition of new antimicrobial families to this backbone.

The data presented shows the presence of cross- and co-resistance among Danish porcine *E. coli*. Such data have not previously been retrieved from the DANMAP. Data presented here clearly indicate the ability of low-classified antimicrobials as ampicillin to uphold resistance to critical important antimicrobials.

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Table 1. Co- and cross-resistance in *E. coli* isolates from 2009-2013 in pigs.

		Group	Tetracyclines	β-lactams			Sulphonamides	Trimethoprim	Aminoglycosides					Amphenicols		Quinolones		Polymyxins
n	**n/N* %**	**ANTIB**	TET	AMP	CTX	CEF	SUL	TRI	STR	SPE	NEO	APR	GEN	CHL	FLO	NAL	CIP	COL
263	34	TET	100	50	1	1	63	44	71	29	10	1	2	8	1	2	2	
205	27	AMP	64	100	2	2	85	64	83	21	11	1	2	9	1	2	2	
5	1	CTX	40	100	100	100	80	60	60	40				20				
5	1	CEF	40	100	100	100	80	60	60	40				20				
252	33	SUL	66	69	2	2	100	63	85	33	12	1	2	12	2	2	2	
170	22	TRI	69	78	2	2	93	100	82	28	11	1	2	11	1	2	2	
320	42	STR	58	53	1	1	67	43	100	46	9	1	2	7	1	1	1	
159	21	SPE	47	28	1	1	53	30	92	100	8	1	1	11	1			
34	4	NEO	76	68			91	53	85	38	100			3				
3	0.4	APR	100	100			67	67	100	33		100	100	33		33	33	
5	1	GEN	80	100			80	80	100	20		60	100	60	40	20	20	
34	4	CHL	59	56	3	3	91	56	68	53	3	3	9	100	12	3	3	
4	1	FLO	50	75			100	50	100	25			50	100	100			
5	1	NAL	100	100			100	80	80			20	20	20		100	100	
5	1	CIP	100	100			100	80	80			20	20	20		100	100	
2	0.3	COL		50														100

* N: total number of resistant isolates

Table 1

Table of co- and cross resistance among 765 *E. coli* of porcine origin isolated in Denmark 2009-2013. Numbers given are in percentage of the total bacteria resistant to antimicrobials given as columns for the individual tested 16 antimicrobials belonging to eight structural families. Total number of resistant are given in column one (n). Percentage of the total amount of tested bacteria are given in column n/N, where N is the total number of tested isolates and n is the number of resistant isolate to the tested antimicrobial. Percentage below 1% is excluded from the Table. Abbreviations: TET (tetracycline), AMP (ampicillin), CTX (cefotaxime), CEF (ceftiofur), SUL (sulfonamide), TRI (trimethoprim), STR (streptomycin), SPE (spectinomycin), NEO (neomycin), APR (apramycin), GEN (gentamicin), CHL (chloramphenicol), FLO (florfenicol), NAL (nalidixic

acid), CIP (ciprofloxacin), COL (colistin). Hatched grey areas indicate higher percentage of co- and cross resistance.

Highlights

- Presenting prevalence of co- and cross-resistance
- Identifying possible co-selection of antimicrobial resistance among tested isolates
- The importance of usage of ampicillin for production animals